

Neonatal Lethal Costello Syndrome and Unusual Dinucleotide Deletion/Insertion Mutations in *HRAS* Predicting p.Gly12Val

Emma M.M. Burkitt-Wright,¹ Lisa Bradley,² Jennifer Shorto,¹ Vivienne P.M. McConnell,² Caroline Gannon,³ Helen V. Firth,⁴ Soo-Mi Park,⁴ Angela D'Amore,⁵ Paul F. Munyard,⁶ Peter D. Turnpenny,⁷ Amanda Charlton,⁸ Meredith Wilson,⁹ and Bronwyn Kerr^{1*}

¹Genetic Medicine, Manchester Academic Health Science Centre, University of Manchester and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK

²Northern Ireland Regional Genetics Service, Belfast, UK

³Department of Pathology, Royal Belfast Hospitals, Belfast, UK

⁴Department of Clinical Genetics, East Anglian Medical Genetics Service, Addenbrooke's Hospital, Cambridge, UK

⁵Department of Neonatology, Addenbrooke's Hospital, Cambridge, UK

⁶Department of Pediatrics, Royal Cornwall Hospital, Truro, UK

⁷Clinical Genetics Department, Royal Devon & Exeter Hospital, Exeter, UK

⁸Department of Pediatric Pathology, Children's Hospital at Westmead, Sydney, New South Wales, Australia

⁹Department of Clinical Genetics, Children's Hospital at Westmead, Sydney, New South Wales, Australia

Received 18 October 2011; Accepted 29 December 2011

De novo heterozygous mutations in *HRAS* cause Costello syndrome (CS), a condition with high mortality and morbidity in infancy and early childhood due to cardiac, respiratory, and muscular complications. *HRAS* mutations predicting p.Gly12Val, p.Gly12Asp, and p.Gly12Cys substitutions have been associated with severe, lethal, CS. We report on molecular, clinical, and pathological findings in patients with mutations predicting *HRAS* p.Gly12Val that were identified in our clinical molecular genetic testing service. Such mutations were identified in four patients. Remarkably, three were deletion/insertion mutations affecting coding nucleotides 35 and 36. All patients died within 6 postnatal weeks, providing further evidence that p.Gly12Val mutations predict a very poor prognosis. High birth weight, polyhydramnios (and premature birth), cardiac hypertrophy, respiratory distress, muscle weakness, and postnatal growth failure were present. Dysmorphism was subtle or non-specific, with edema, coarsened facial features, prominent forehead, depressed nasal bridge, anteverted nares, and low-set ears. Proximal upper limb shortening, a small bell-shaped chest, talipes, and fixed flexion deformities of the wrists were seen. Neonatal atrial arrhythmia, highly suggestive of CS, was also present in two patients. One patient had congenital alveolar dysplasia, and another, born after 36 weeks' gestation, bronchopulmonary dysplasia. A rapidly fatal disease course, and the difficulty of identifying subtle dysmorphism in neonates requiring intensive care, suggest that this condition remains under-recognized, and should enter the differential diagnosis for very sick infants with a range of clinical problems including cardiac

How to Cite this Article:

Burkitt-Wright EMM, Bradley L, Shorto J, McConnell VPM, Gannon C, Firth HV, Park S-M, D'Amore A, Munyard PF, Turnpenny PD, Charlton A, Wilson M, Kerr B. 2012. Neonatal lethal Costello syndrome and unusual dinucleotide deletion/insertion mutations in *HRAS* predicting p.Gly12Val. *Am J Med Genet Part A* 158A:1102–1110.

hypertrophy and disordered pulmonary development. Clinical management should be informed by knowledge of the poor prognosis of this condition. © 2012 Wiley Periodicals, Inc.

Grant sponsors: Wellcome trust and UK National Institute for Health Research's Manchester Biomedical Research Centre.

The authors have no competing interests to declare.

*Correspondence to:

Dr. Bronwyn Kerr, MBBS, FRACP, Genetic Medicine, 6th Floor St Mary's Hospital, Central Manchester Foundation Trust, Oxford Road, Manchester M13 9WL, UK. E-mail: bronwyn.kerr@cmft.nhs.uk

Published online 11 April 2012 in Wiley Online Library (wileyonlinelibrary.com).

DOI 10.1002/ajmg.a.35296

Key words: Costello syndrome; *HRAS*; neonatal cardiomyopathy; congenital alveolar dysplasia; dinucleotide insertion/deletion mutation

INTRODUCTION

Costello syndrome (CS) is a rare condition which arises due to heterozygous germline mutations in *HRAS*, resulting in expression of constitutively active *HRAS* proteins [Aoki et al., 2005]. Much the commonest of these is p.Gly12Ser, which accounts for approximately 80% of diagnosed cases [Kerr et al., 2006]. Common clinical features include prenatal overgrowth and polyhydramnios, severe postnatal failure to thrive, short stature, developmental delay, congenital heart disease, and cardiomyopathy [Kerr, 2009]. Whilst CS often has a relatively homogenous phenotype, both milder and more severe phenotypes are now recognized, which often arise due to less common mutations [Kerr et al., 2006; van der Burgt et al., 2007; Gripp et al., 2011]. Severe CS and congenital myopathy with excess of muscle spindles, a variant manifestation of the same condition, have been described in association with mutations predicting amino acid substitutions p.Gly12Val [Aoki et al., 2005; van der Burgt et al., 2007], p.Gly12Asp and p.Gly12Cys [Lo et al., 2008], but with only a very few patients in the literature. We present here a series of four further patients with a variety of *HRAS* mutations predicting p.Gly12Val. These p.Gly12Val mutations, rare in the germline but much more commonly observed in cancers, were associated with a severe presentation lethal in the first weeks of life in all four patients.

MATERIALS AND METHODS

Exons 2 to 6 of *HRAS* were sequenced and analyzed on an ABI 3730 sequencer, within the molecular diagnostic service offered by Manchester Regional Genetics Laboratory. Clinical and histopathological details of patients in whom mutations predicting p.Gly12Val were identified are described below and summarized in Table I.

RESULTS

Patient 1

This boy was the second child of healthy unrelated Irish parents, his mother being 28 and father 33 years of age. His older sister was stillborn at 41 weeks' gestation weighing 2,750 g (9th centile), with no dysmorphic features. No postmortem was performed in her.

Fetal macrosomia was identified in the proband at 34 weeks' gestation. Maternal HbA_{1c}, glucose tolerance test and a congenital viral infection screen were normal. He was delivered by cesarean at 36⁺⁶ weeks' gestation for sudden onset polyhydramnios and reduced fetal movements, weighing 3,325 g (75th centile), with an OFC of 38.2 cm (>99.6th centile). Apgar scores were good (8¹ and 9⁵), but he quickly became ventilator dependent with severe central hypotonia. Obstructing vocal cord granulomas required carbon dioxide laser treatment on day 39. He died on day 42 of bronchopneumonia.

Dysmorphic features (Fig. 1a,b) included frontal bossing, a box-shaped face, depressed nasal bridge with anteverted alae nasi, large dysplastic ears, abundant loose skin around the mandible, a short,

TABLE I. Summary of Clinical Features of Patients With p.Gly12Val Mutations

Feature	Present in
Severe polyhydramnios	5/5
Delivery before 37 weeks' gestation	5/5
Birth weight on above 90th centile	4/5
Birth OFC on or above 90th centile	5/5
Coarsening of facial features	5/6
Unusual hand position	5/6
Unusual foot position	4/5
Short neck	5/6
Narrow thorax and protuberant abdomen	3/4
High cryptorchidism	2/2
Cardiac hypertrophy	6/6
Structural findings on echocardiography (abnormal pulmonary outflow tract:1; patent foramen ovale:1)	2/4
Cardiac arrhythmia	2/4
Ventilator dependence	5/6
Hepatosplenomegaly	2/5
Death before 6 weeks postnatal age ^a	5/6

^aThe other previously published patient with a p.Gly12Val mutation also had a lethal course, surviving to 18 months of age [Aoki et al., 2005].

fleshy neck, small thorax with widely spaced nipples, upper limb rhizomelic shortening, peripheral arthrogyriposis with slender digits, bilateral undescended testes, and talipes equinovarus. Blood and skin karyotypes showed 46, XY. Subtelomere microdeletion/duplication multiplex ligation probe amplification (MRC Holland kit PO36E1), *RAPSN* analysis, extensive metabolic workup, magnetic resonance imaging of brain and abdomen, initial echocardiogram, bone age, bone density, and skeletal survey all showed normal results. Repeat echocardiography on day 36 demonstrated biventricular hypertrophy with a small pericardial effusion.

Postmortem examination revealed length and head circumference on the 25th centile, but weight below the 9th, despite apparent excessive subcutaneous tissue of the limbs, face, and neck. Heart weight was 34 g (expected: 20 g), biventricular and septal hypertrophy (Fig. 2a) with mild interstitial edema were present, but no fibrosis or myofibrillar disarray. Other muscles were firm and bulky, especially the diaphragm (Fig. 2b,c). Evidence of bronchopneumonia and healing bronchopulmonary dysplasia confirmed the cause of death. The pancreas showed increased islet cell size and number, whilst the thymus was small and atrophic. Immature cryptorchid testes were well above the pelvic rim. The brain appeared structurally normal, but weighed 602 g (expected: 413 g). Radiographs and the rest of the internal examination were normal, with no further histological abnormalities evident. The diagnosis of CS was only established some time after the baby's death, when he was presented at an international dysmorphology meeting.

Patient 2

This baby girl was the first child of unrelated Australian parents, a 27-year-old mother and 31-year-old father. Severe polyhydramnios



FIG. 1. Clinical appearance of patients. a,b: Patient 1, appearance on day 2 of life. Note overall fleshy/edematous appearance to face, trunk and limbs, rhizomelic shortening with marked circumferential skin folds in the arms and peripheral edema. Dysmorphic facial features including low-set fleshy ears with overfolded helices and upturned lobes, short nose with depressed nasal bridge and anteverted nares, and micrognathia are present. c–f: Patient 3. c: A deep plantar crease is seen in the foot, which is held in equinovarus. d: Macrosomia, protuberant abdomen, and a small, narrow thorax with widely spaced nipples are shown. Flexed wrists with hands held in ulnar deviation are also seen. e,f: Coarse facial appearance, a prominent forehead, depressed nasal bridge with anteverted nares, and low-set, fleshy ears, are shown.

was noted at 25 weeks, requiring amnioreduction of 2 L at 26 weeks because of threatened premature labor. Ultrasound at 27 weeks showed persistent mild polyhydramnios, short limbs, prominent abdomen, and bell-shaped chest. Fetal MRI showed mild ventricular dilatation. Steroids were administered at 29 weeks, and emergency cesarean delivery was necessary at 30 weeks for a non-reassuring CTG. Apgar scores were 4¹ and 7⁵; she was intubated and ventilated from birth and a single dose of surfactant given. Her birth weight was 1,926 g (>97th centile), length 25–50th centile and OFC 90th centile.

Marked generalized edema, a bell-shaped chest, prominent abdomen, and rhizomelic arm shortening were evident postnatally, with coarse facies, high prominent forehead, depressed nasal bridge, pursed lips, microretrognathia, a “double chin,” excess nuchal skin, edematous fingers, and rocker bottom feet. Her hands were flexed at the wrists and extended at the metacarpophalangeal joints, with thumbs adducted and fingers flexed, but without contractures. There was no organomegaly, ascites, or pleural effusion. Edema resolved over the first week and her weight decreased to the 50th centile. Cranial ultrasound showed mild ventricular dilatation and bilateral grade I intraventricular hemorrhage.

A skeletal dysplasia was initially suspected, but skeletal survey revealed no additional abnormalities. Abdominal ultrasound, liver

function tests, EEG, transferrin isoforms, very long-chain fatty acids, white cell enzymes, karyotype, urinary glycosaminoglycans, amino and organic acids, carnitines and acylcarnitine profile were also within normal limits. She developed mild hyponatremia and maximum serum bilirubin was 219 $\mu\text{mol/L}$.

Requirements for high ventilatory pressures and P_iO_2 persisted, with an inability to tolerate ventilatory rates below 40 per min. Chest X-rays showed non-specific hazy opacities and migrating lung collapses, and the clinical course suggested pulmonary hypoplasia/dysplasia. At 3 weeks, three episodes of supraventricular tachycardia occurred in 24 hr, two requiring cardioversion, the other was brief and resolved spontaneously. Subsequent ECG showed right axis deviation, borderline left bundle branch block and T wave inversion in chest leads. Echocardiogram revealed a patent foramen ovale and mild asymmetric septal hypertrophy. Clinical genetic assessment revealed deep palmar and plantar creases and typical hand positioning suggestive of severe neonatal CS. Given her poor prognosis, persisting high ventilatory requirements and features of severe pulmonary dysplasia, the managing team and parents jointly decided to discontinue mechanical ventilation on day 36, and she died soon afterwards.

At postmortem, body weight was 5th centile, crown-rump and foot lengths <3rd centile, and OFC 10th centile (for 35 weeks’

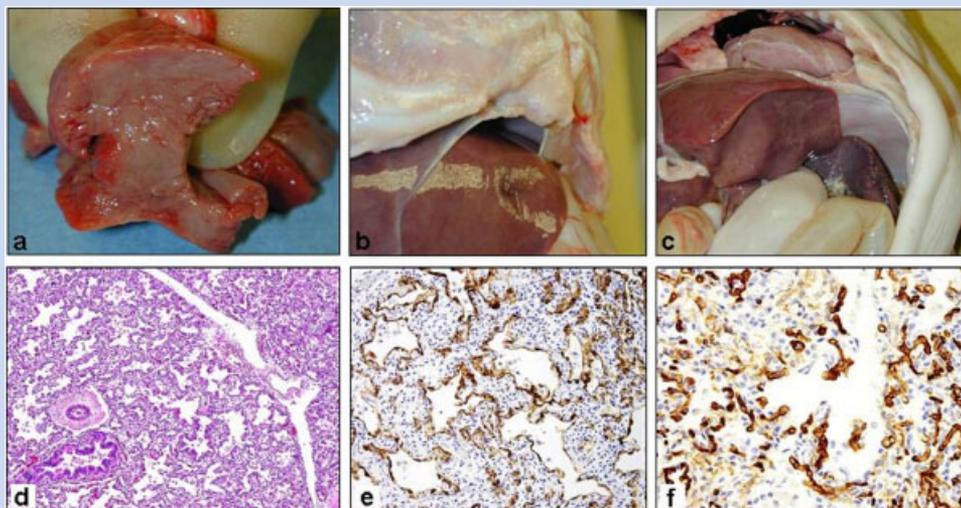


FIG. 2. Postmortem anatomy and histology. **a:** Macroscopic appearance of coronal section through the heart, showing hypertrophic cardiomyopathy, with particularly marked septal hypertrophy. **b,c:** View into chest and abdominal cavity. Note generalized pallor of muscles and thickening of diaphragm. **d:** Delayed lung development for 35 weeks' gestation (adjusted), similar to canalicular phase, is shown. The pulmonary artery and bronchiole travel together (left), and the pulmonary vein (right) is normally positioned in the interlobular septum. Hematoxylin and eosin stain, original magnification 200 \times . **e:** Cytokeratin (brown chromogen) marks the alveolar lining cells, demonstrating an excess of stroma and too little airspace. Cytokeratin AE1/3 immunoperoxidase, hematoxylin counterstain, original magnification 200 \times . **f:** CD34 (brown chromogen) marks the capillaries. Capillary apposition and density is not decreased. CD34 immunoperoxidase, hematoxylin counterstain, original magnification 400 \times .

gestation). Increased subcutaneous tissue of the face and neck, lax skin of the trunk and limbs, deep palmar and plantar creases and perianal papillomata were present. Consent for internal examination was limited to lung biopsy. The right upper and middle lobes were removed and appeared heavy, solid, and airless. Histology of formalin-fixed sections (Fig. 2d–f) showed a diffuse developmental disorder: development was arrested in the canalicular stage, normally seen at 17–27 weeks' gestation [Langston and Dishop, 2009]. The pulmonary veins were not misaligned, and capillary density and apposition were normal, thus the features were not of alveolar capillary dysplasia [Melly et al., 2008]. Delayed maturation was in keeping with congenital alveolar dysplasia (CAD) [MacMahon, 1948]. The radial alveolar count was normal (3; mean expected for 32–35 weeks: 3.2 ± 0.9 [Emery and Mithal, 1960]), and there was no lymphangiectasia.

Patient 3

This baby girl was the product of an IVF/ICSI pregnancy using a donor ovum from a healthy 29-year-old British Caucasian woman, due to failure of spontaneous conception by her 48-year-old mother and 61-year-old father. The parents had a previous naturally conceived child with non-disjunctional trisomy 21, born when the mother was 42 years old.

Fetal macrosomia was identified at 20 weeks' gestation. By 33⁺⁴ weeks, very marked polyhydramnios was present, with head and abdominal circumferences well above the 97th centile, but femoral lengths below the 50th centile, and evidence of gross hepatomegaly. The kidneys were not visualized, but neither an abdominal wall

defect nor macroglossia were detected. Gestational diabetes was diagnosed at 24 weeks' gestation, but was well controlled with insulin and diet (HbA_{1c} 5.4%), and hence considered unlikely to be a significant contributor to the abnormalities identified on ultrasound.

Emergency cesarean was undertaken for fetal distress after spontaneous onset of labor at 36⁺⁴ weeks. Meconium stained liquor was present, and there was no respiratory effort at birth. Apgar scores were 4¹ and 6⁵. Marked macrosomia was present, with birth weight (4,070 g) and OFC (37.3 cm) both greatly above the 99.6th centile. She was blue and hypotonic, with a small thorax, and required resuscitation, intubation, and ventilation. Marked respiratory distress necessitated ongoing ventilatory support with high oxygen requirements and a diaphragmatic breathing pattern reflecting laryngomalacia and hypotonia. A diagnosis of CS was considered, given her macrosomia (Fig. 1c,d), disproportionately large head with prominent forehead, depressed nasal bridge with anteverted nares, coarse facial appearance and low set, fleshy ears (Fig. 1e,f). Her protuberant abdomen, narrow thorax with widely spaced nipples, deep palmar and plantar creases, deep set nails and bilateral talipes equinovarus, with wrists held in fixed flexion, were also in keeping with this.

On day 6, she developed a tachycardia of 200 beats/min. ECGs showed abnormal P wave morphology with multifocal atrial tachycardia, or sinus tachycardia with intermittent atrial tachycardia. Echocardiography (having been normal on day 2) then showed ventricular hypertrophy, massive tricuspid regurgitation and an abnormal pulmonary outflow tract. Subsequent echocardiograms showed worsening biventricular concentric hypertrophy, particularly

affecting the right ventricle, and evidence of secondary pulmonary hypertension. Cardiomegaly and features of pulmonary edema were also seen on chest X-ray. Skeletal survey confirmed a narrow ribcage but no other abnormalities, cranial ultrasound scan was normal, and karyotype was 46, XX. Hepatosplenomegaly persisted postnatally, with fluctuating conjugated jaundice and raised transaminases after initially normal liver function tests. She also developed sepsis, anemia, and hyponatremia with high urinary sodium losses and elevated urinary vanillylmandelic acid. In view of increasing ventilatory requirements and poor prognosis, the decision was reached with her parents to discontinue intensive management and she died shortly afterwards on day 39. No postmortem examination was conducted.

Patient 4

This girl was the first child of non-consanguineous British Caucasian parents, the father and mother aged 24 and 22 years, respectively, at the time of conception. Polyhydramnios was noted on ultrasound at 27 weeks' gestation, though this was not seen at 28 weeks, when premature labor occurred. Two doses of dexamethasone were administered before spontaneous vaginal delivery of the baby, who weighed 1,377 g (75th–91st centile). Meconium was present and she made no spontaneous respiratory effort. She was intubated at 7 min and received surfactant (Curosurf). Respiratory distress syndrome was treated with mechanical ventilation until day 8. Reintubation and ongoing ventilation became necessary on day 10. Chest X-ray showed persistent right upper lobe collapse. Echocardiography on day 15 showed a very thick intraventricular septum and thick ventricular walls, and hypertrophic cardiomyopathy was diagnosed. Cranial ultrasonography demonstrated bilateral periventricular flare. She remained parenterally fed, became increasingly difficult to ventilate, and died on day 17.

Subtle dysmorphic features had been noted, including a short neck with possible webbing, widely spaced nipples and rhizomelic shortening of the limbs. Karyotype was 46, XX and *PTPN11* mutation analysis was normal. Eight years later, the family was referred to the genetics service for investigation of a male sibling with developmental delay and dysmorphic features (for whom a diagnosis is still not established). On the strength of a family photograph of the patient, taken after withdrawal of treatment, the diagnosis of CS was considered and her stored DNA was sent for *HRAS* mutation analysis.

MOLECULAR RESULTS

Heterozygous missense mutations in *HRAS* were identified in all four patients, each predicting p.Gly12Val at the protein level. A variety of nucleotide changes were identified, 3 of the 4 arising as deletion/insertion mutations of nucleotides 35 and 36 (Table II). Of note, no isolated substitutions of nucleotide 36 (any of which would be synonymous) have been identified in any other sample of 213 submitted for *HRAS* sequencing analysis in our laboratory, and nor is any substitution at this base a recognized polymorphism.

De novo occurrence of the mutations was confirmed by absence of the changes in parental DNA, where this was available: the mutation was confirmed to be absent from lymphocyte DNA of

TABLE II. Germline Mutations Predicting p.Gly12Val in Patients With Severe Costello Syndrome

Patient	Nucleotide substitution
1	c.35_36delinsTA
2	c.35_36delinsTT
3	c.35_36delinsTT
4	c.35G > T
Aoki et al. [2005] [Patient COS37]	c.35_36delinsTT
van der Burgt et al. [2007] [Patient 1]	c.35_36delinsTT

the parents of Patient 1 (Fig. 3) and Patient 2. The latter couple also opted to have prenatal testing for gonadal mosaic risk in a subsequent pregnancy, with normal results. The parents of other patients in this series did not pursue genetic testing on their own accounts in view of low recurrence risks and their personal circumstances.

The preponderance of dinucleotide deletion/insertion mutations identified in this series is remarkable, as such mutations are exceedingly rare both in other germline disorders and also in cases of somatic mutation, such as those that occur in cancer. The COSMIC database of somatic alterations in cancer (<http://www.sanger.ac.uk/perl/genetics/CGP/cosmic>), as of 6th October 2011, included 749 *HRAS* mutations, identified in 21,905 tumor samples tested. Of these, 453 affected codon 12. These are shown in Figure 4, and of note none were dinucleotide deletion/insertions. Similar mutations have, however, been very rarely identified affecting codon 61 of *HRAS* (in 6/21905 tumor samples), and also with extreme rarity in other RAS genes in cancers, for example 54 such mutations altering codon 12 of *KRAS* have been included in COSMIC, in comparison to 17,490 point mutations altering this codon (in a total of 92,270 tumor samples included at October 6, 2011).

DISCUSSION

Clinical Presentation of p.Gly12Val Mutations

This series demonstrates that a variety of clinical manifestations of severe CS are present in infants with *HRAS* p.Gly12Val mutations, but all of these patients had ultimately similar, lethal, outcomes. There are only two patients in the literature with similar mutations, one described as having severe neonatal CS [Aoki et al., 2005], and one with congenital myopathy with excess of muscle spindles [van der Burgt et al., 2007, initially reported by de Boode et al., 1996]. Both died in the first 2 years of life, at 18 months and 3 weeks, respectively. Severe CS, lethal in the neonatal period, has also been described in association with other *HRAS* mutations of codon 12, both as an unusual manifestation of the commonest CS allele, p.Gly12Ser, and with rare p.Gly12Asp, p.Gly12Cys, and p.Gly12Glu alleles [Kerr et al., 2006; Lo et al., 2008], which similarly are also identified with greater frequency in tumors (Fig. 4).

Given the subtle or non-specific facial dysmorphism observed in many of these patients, the variable presentations (such as congenital skeletal myopathy, cardiomyopathy, or pulmonary

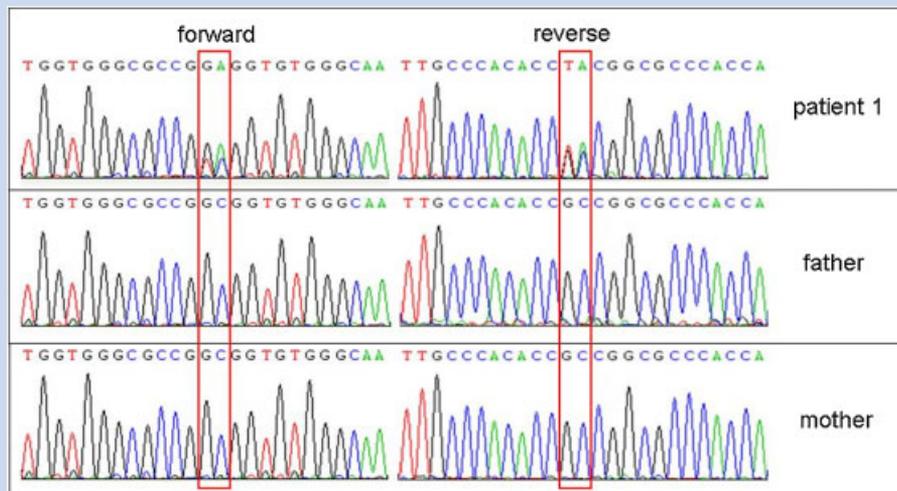


FIG. 3. DNA sequencing demonstrates a de novo double substitution. Forward and reverse sequencing traces of Patient 1, his father and mother. The heterozygous deletion/insertion c.35_36delinsTA in the patient, and its absence from the parents, is indicated in the boxes.

hypoplasia), and the rapidly fatal course, this may be a condition that is currently under-diagnosed. Two of the four patients described here were not diagnosed until some time after their deaths. The extensive battery of investigations undertaken in patients such as Patient 1 in this series further emphasizes this diagnostic difficulty. The possibility of a severe mutation in *HRAS* is important to consider in differential diagnosis in view of the apparently extremely poor prognosis. This could also be important information for parents and obstetricians regarding potential prenatal diagnosis of a mutation predicting *HRAS* p.Gly12Val in an affected fetus, as knowledge of the likely adverse outcome (even compared to the significant morbidity and mortality seen in association with *HRAS* p.Gly12Ser) could inform decisions regarding management of the pregnancy.

The presence of subtle or non-specific dysmorphic features in affected individuals may be difficult to identify in critically ill neonates, where prematurity, edema, and intensive care interventions could each hinder comprehensive examination. It is also possible that there may be significant prenatal lethality associated with severe *HRAS* mutations, during either embryonic or fetal development, which would further hinder recognition of the full phenotypic spectrum associated with such alleles.

Histopathological Implications of Germline p.Gly12Val Mutations

Despite the key role that *HRAS* p.Gly12Val can play in the genesis of cancer (*HRAS* being the first identified oncogene [Parada et al., 1982]), no malignancies have been observed in individuals bearing such a mutation in their germline. This is likely due to the severity of the resulting phenotype, rendering it lethal before malignant tumors have a chance to develop. The inability of oncogenic mutant Ras alone to cause tumorigenesis has been known for many years

[Land et al., 1983], and is further borne out by the fact that not all individuals with CS develop cancer. The lack of likelihood of acquisition of sufficient other oncogenic mutations in the short lifespan of individuals with heterozygous mutations in *HRAS* predicting p.Gly12Val makes the development of cancers in these babies unlikely. The occurrence of vocal cord granulomas in Patient 1 in this series could be regarded as a recognized sequela of prolonged ventilation, but the possibility exists that mutation in *HRAS* could be a contributing factor to this unusual complication of endotracheal intubation, particularly as a large tracheal polyp was identified in a patient with lethal CS due to a p.Gly12Glu mutation in a previous series [Patient 11, Kerr et al., 2006].

The single previous report of lung histopathology in neonatal CS described “pulmonary dysplasia such as alveolar capillary dysplasia” [Lo et al., 2008]. Patient 2’s lung histology showed CAD, which has not previously been described in neonatal CS. CAD was first described in 1948 [MacMahon, 1948], but there are few subsequent reports. CAD is less well known than alveolar capillary dysplasia (ACD), which shows misalignment of pulmonary veins [Melly et al., 2008]. The phenotype of CAD is of ventilator-dependent respiratory failure, and the histology is of arrest in the canalicular/early sacular stage. The pattern is of “too much stroma, too little airspace” (as seen in Fig. 2e), with widened airspace walls, and without fibroplastic appearances. It is thought likely that CAD and ACD demonstrate overlaps both in clinical presentation and histology [Melly et al., 2008], and the reporting of CAD in Patient 2 in this series, and possible ACD in a previously reported patient with lethal CS [Patient 1, Lo et al., 2008], may reflect this.

Myocardial hypertrophy appears to be a consistent feature of patients with severe CS, and though the degree has been variable, its presence may be a useful diagnostic indicator, and should raise the suspicion of an *HRAS* mutation. This and other clinical features

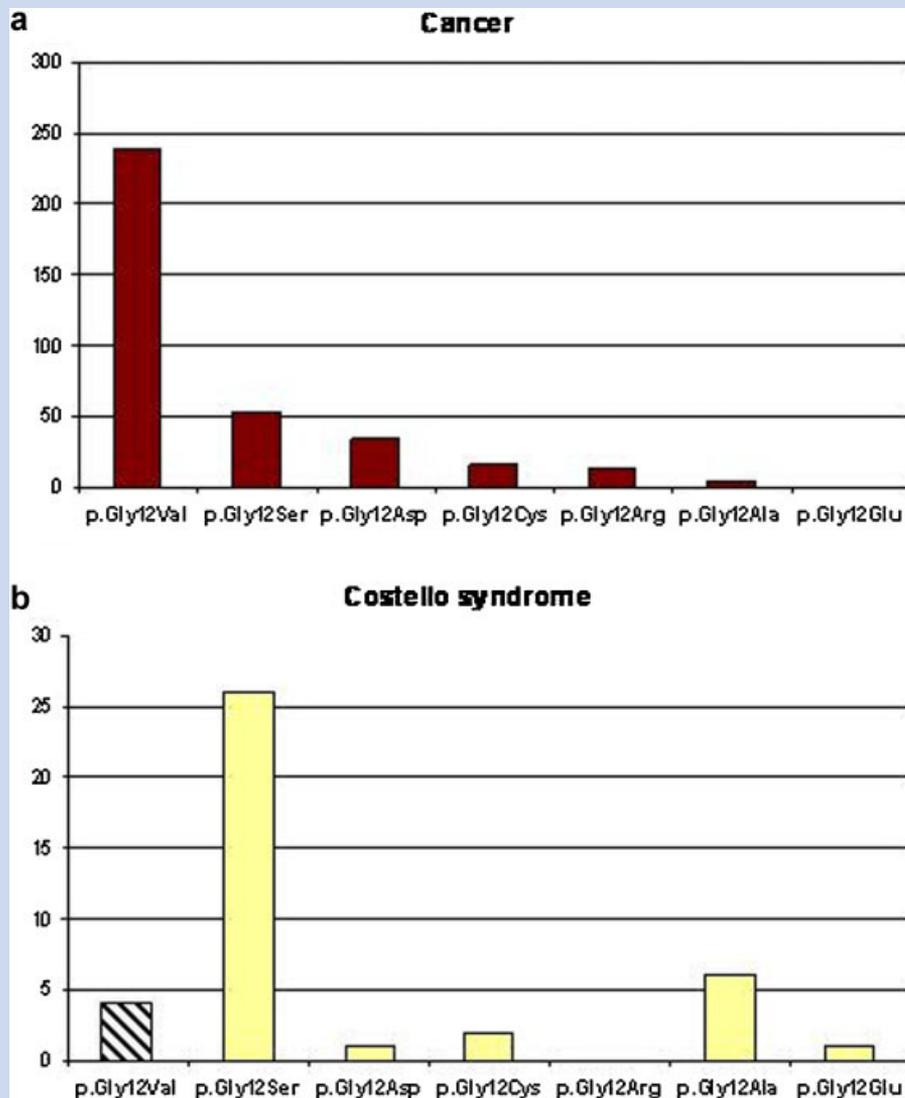


FIG. 4. Comparison of *HRAS* mutations affecting codon 12 identified in Costello syndrome and in cancers. **a:** *HRAS* codon 12 mutations identified in cancers (recorded in the COSMIC database) demonstrate a high preponderance of substitutions predicting p.Gly12Val. **b:** *HRAS* codon 12 mutations in patients with CS tested in the Manchester Regional Genetics Laboratory. The p.Gly12Val mutations described in this report are shown hatched. Note the high preponderance of p.Gly12Ser mutations, with much lower numbers of other substitutions, in contrast to the mutations identified in cancers.

common to all known patients with mutations predicting p.Gly12Val are shown in bold in Table I. The finding of atrial arrhythmia in two of the four patients, as is also commonly described in other patients with CS, as well as other disorders of the RAS-MAPK pathway such as Noonan syndrome due to *RAF1* mutations [Kobayashi et al., 2010], emphasizes the importance of such cardiac phenotypes in assisting recognition of the presence of a neurocardiofaciocutaneous disorder in infants with multiple medical problems. The identification of thymic atrophy in Patient 1 is reminiscent of that reported in a postmortem of an individual affected with cardio-facio-cutaneous (CFC) syndrome [Manci et al., 2005], and also of the phenotype of the B-Raf^{d.SLV600E} mouse

model of CFC syndrome [Urošević et al., 2011], but at present both the pathogenesis and effects of these abnormalities are unclear, and they remain of uncertain significance.

Implications for Mutational Mechanisms: An Unexplained Preponderance of Dinucleotide Deletion/Insertion Mutations in *HRAS* Causing Severe CS

The very high proportion of mutations identified to cause severe CS that arise as dinucleotide deletion/insertions remains unexplained.

Five of the total of six germline mutations predicting p.Gly12Val identified to date, and also the single identified mutation predicting p.Gly12Glu, are of this nature [Kerr et al., 2006]. This proportion is in stark contrast to the extreme rarity with which dinucleotide deletion/insertion mutations in Ras genes have been observed in cancers, compared to the enormous number of point mutations. It is also notable that this form of mutation appears extremely rare in other genetic disorders: very few patients with dinucleotide deletion/insertion mutations have been described in the literature. A single patient with a *PTPN11* mutation, c.1471_1472delinsTT [Schuettpelez et al., 2009], of many hundreds known, and a single patient with a *SOS1* mutation, c.1300_1301delinsAA [Lepri et al., 2011] are examples. Both of these mutations affect codons that are recurrently mutated in Noonan syndrome, and like the *HRAS* mutations that cause CS and occur in cancer, appear to result in gain-of-function alleles. Similar dinucleotide mutations have also, rarely, been described in *FGFR2*, resulting in Apert or Pfeiffer syndrome [Oldridge et al., 1997; Kan et al., 2002]. A selective advantage for spermatogonia bearing activating mutations in genes including *HRAS* and *FGFR2* has been demonstrated [Goriely et al., 2009], but the dramatically elevated proportion of germline mutations predicting *HRAS* p.Gly12Val that are dinucleotide deletion/insertions, as opposed to c.35G>T point mutations, suggests that the mechanisms by which these two classes of mutation are generated, and perhaps their effects upon human development, might be distinct, and that this area requires further investigation.

CONCLUSION

Mutations predicting severely activating *HRAS* proteins such as p.Gly12Val are a recognized cause of severe CS, which frequently has a presentation that is lethal in the neonatal period, though the common CS p.Gly12Ser mutation has also been described occasionally in similarly severely affected individuals. Recognition of this condition may be hindered by the extreme illness of these babies, and their rapid demise. Consideration should be given to the possibility of severe CS as the cause of a range of presentations in very sick neonates, especially those born prematurely as a consequence of extreme polyhydramnios. Identification of this condition in utero may also be possible in certain cases, and could be confirmed by prenatal diagnosis. Hypertrophic cardiomyopathy appears to be a consistent feature, along with hypotonia due to skeletal muscle weakness. Other features of CS, such as atrial arrhythmia, or suggestive dysmorphic features, may also be present.

The unusual observation of dinucleotide deletion/insertions as a very high proportion of mutations causing severe CS, and its contrast with the mutational spectrum observed in cancers, suggest that differences may exist in the mutational mechanisms at work in the two contexts.

ACKNOWLEDGMENTS

EBW is a Wellcome Trust clinical research training fellow, supported by the UK National Institute for Health Research's Manchester Biomedical Research Centre.

REFERENCES

- Aoki Y, Niihori T, Kawame H, Kurosawa K, Ohashi H, Tanaka Y, Filocamo M, Kato K, Suzuki Y, Kure S, et al. 2005. Germline mutations in *HRAS* proto-oncogene cause Costello syndrome. *Nat Genet* 37:1038–1040.
- de Boode WP, Semmekrot BA, ter Laak HJ, van der Burgt CJAM, Draaisma JMT, Lommen EJP, Sengers RCA, van Wijk-Hoek JM. 1996. Myopathology in patients with a Noonan phenotype. *Acta Neuropathol* 92:597–602.
- Emery JL, Mithal A. 1960. The number of alveoli in the terminal respiratory unit of man during late intrauterine life and childhood. *Arch Dis Child* 35:544–547.
- Goriely A, Hansen RM, Taylor IB, Olesen IA, Jacobsen GK, McGowan SJ, Pfeifer SP, McVean GA, Rajpert-De Meyts E, Wilkie AO. 2009. Activating mutations in *FGFR3* and *HRAS* reveal a shared genetic origin for congenital disorders and testicular tumors. *Nat Genet* 41:1247–1252.
- Gripp KW, Hopkins E, Sol-Church K, Stabley DL, Axelrad ME, Doyle D, Dobyns WB, Hudson C, Johnson J, Tenconi R, et al. 2011. Phenotypic analysis of individuals with Costello syndrome due to *HRAS* p.G13C. *Am J Med Genet A* 155A:706–716.
- Kan SH, Elanko N, Johnson D, Cornejo-Roldan L, Cook J, Reich EW, Tomkins S, Verloes A, Twigg SR, Rannan-Eliya S, et al. 2002. Genomic screening of fibroblast growth-factor receptor 2 reveals a wide spectrum of mutations in patients with syndromic craniosynostosis. *Am J Hum Genet* 70:472–486.
- Kerr B. 2009. The Clinical phenotype of Costello syndrome. In: Zenker M, editor. Noonan syndrome and related disorders. *Monogr Hum Genet*, vol 17. Basel: Karger. pp 83–93.
- Kerr B, Delrue M-A, Sigaudy S, Perveen R, Marche M, Burgelin I, Stef M, Tang B, Eden OB, O'Sullivan JO, et al. 2006. Genotype-phenotype correlation in Costello syndrome: *HRAS* mutations analysis in 43 cases. *J Med Genet* 43:401–405.
- Kobayashi T, Aoki Y, Niihori T, Cavé H, Verloes A, Okamoto N, Kawame H, Fujiwara I, Takada F, Ohata T, et al. 2010. Molecular and clinical analysis of *RAF1* in Noonan syndrome and related disorders: Dephosphorylation of serine 259 as the essential mechanism for mutant activation. *Hum Mutat* 31:284–294.
- Land H, Parada LF, Weinberg RA. 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two co-operating oncogenes. *Nature* 304:596–602.
- Langston C, Dishop MK. 2009. Diffuse lung disease in infancy: A proposed classification applied to 259 diagnostic biopsies. *Pediatr Dev Pathol* 12:421–437.
- Lepri F, De Luca A, Stella L, Rossi C, Baldassarre G, Pantaleoni F, Cordeddu V, Williams BJ, Dentici ML, Caputo V, et al. 2011. *SOS1* mutations in Noonan syndrome: Molecular spectrum, structural insights on pathogenic effects, and genotype-phenotype correlations. *Hum Mutat* 32:760–772.
- Lo IF, Brewer C, Shannon N, Shorto J, Tang B, Black G, Soo MT, Ng DK, Lam ST, Kerr B. 2008. Severe neonatal manifestations of Costello syndrome. *J Med Genet* 45:167–171.
- MacMahon HE. 1948. Congenital alveolar dysplasia of the lungs. *Am J Pathol* 24:919–931.
- Manci EA, Martinez JE, Horenstein MG, Gardner TM, Ahmed A, Mancao MC, Gremse DA, Gardner DM, Nimityongskul P, Maertens P, et al. 2005. Cardiofaciocutaneous syndrome (CFC) with congenital peripheral neuropathy and nonorganic malnutrition: An autopsy study. *Am J Med Genet* 137A:1–8.
- Melly L, Sebire NJ, Malone M, Nicholson AG. 2008. Capillary apposition and density in the diagnosis of alveolar capillary dysplasia. *Histopathology* 53:450–457.

- Oldridge M, Lunt PW, Zackai EH, McDonald-McGinn DM, Muenke M, Moloney DM, Twigg SR, Heath JK, Howard TD, Hoganson G, et al. 1997. Genotype-phenotype correlation for nucleotide substitutions in the IgII-IgIII linker of FGFR2. *Hum Mol Genet* 6:137–143.
- Parada LF, Tabin CJ, Shih C, Weinberg RA. 1982. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. *Nature* 297:474–478.
- Schuettpelz LG, McDonald S, Whitesell K, Desruisseau DM, Grange DK, Gurnett CA, Wilson DB. 2009. Pilocytic astrocytoma in a child with Noonan syndrome. *Pediatr Blood Cancer* 53:1147–1149.
- Urosevic J, Sauzeau V, Soto-Montenegro ML, Reig S, Desco M, Burkitt Wright EM, Cañamero M, Mulero F, Ortega S, Bustelo XR, et al. 2011. Constitutive activation of B-Raf in the mouse germ line provides a model for human cardio-facio-cutaneous syndrome. *Proc Natl Acad Sci USA* 108:5015–5020.
- van der Burgt I, Kupsky W, Stassou S, Nadroo A, Barroso C, Diem A, Kratz CP, Dvorsky R, Ahmadian MR, Zenker M. 2007. Myopathy caused by HRAS germline mutations: Implications for disturbed myogenic differentiation in the presence of constitutive HRas activation. *J Med Genet* 44:459–462.